RESTRICTED REJOINING OF CHROMOSOMAL SUBUNITS IN ABERRATION FORMATION: A TEST FOR SUBUNIT DISSIMILARITY

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Abstract.—The chromosomal subunits that segregate from each other during chromosome replication are shown to be uniquely different. An analysis of tritiated thymidine labeled chromosomal subunits in dicentric chromosomes, generated by isolocus breakage followed by proximal reunion, shows that the end-to-end association of the subunits is not random but is strictly preferential. The data suggest that the functional subunit in the formation of these particular chromosomal aberrations might be single polynucleotide chains of deoxyribonucleic acid, although other molecular species cannot be disregarded. The suggestion that the DNA is the molecule that is involved is based on the fact that the two chains of the DNA double helix exhibit reverse polarity and this property predicts the observed autoradiographic patterns.

The semiconservative segregation of chromosomal deoxyribonucleic acid (DNA), first demonstrated by Taylor, Woods, and Hughes,[1] is a well-established fact of cytogenetics. It is equally well established that although sister chromatids show this semiconservative segregation of the isotope at the second mitosis after incorporation of tritiated thymidine, they also contain many switches of label from one chromatid to the other. Taylor[2] used these label switches, or sister-chromatid exchanges, to demonstrate that the segregating subunits of the chromosome had directional polarity and were, in all probability, single-stranded DNA.

Taylor argued that if a sister-chromatid exchange were to occur in a chromosome after incorporation of isotope and if subunits were restricted, by directional polarity, in the way they could rejoin, both descendants (daughters) of the chromosome would contain an identical label switch at the second postlabeling mitosis. If both daughters were conserved in one cell by the induction of polyploidy, the original exchange would thus appear as a twin exchange. Any sister-chromatid exchange occurring in the second cell cycle after isotope incorporation would be restricted to that particular chromosome and would appear as a single exchange at the second postlabeling colchicine metaphase. The restriction of directional polarity coupled with the induced doubling of chromosome number leads to a ratio of 1 twin:2 single exchanges in the tetraploid cells of the second postlabeling mitosis. Taylor obtained some data compatible with this ratio and was able to show that other data which deviated from this expectation were explicable in terms of an effect of colchicine on exchange frequency.[2]

Subsequent analyses[3–6] have realized varying ratios of twin:single exchange events and have led various authors to question the supposition that chromo-

somal subunits involved in exchange actually have directional polarity, and, by implication, whether these subunits are the polynucleotide chains of a DNA molecule.

Further doubts as to the validity of the twin:single ratio as a basis for conclusions concerning the nature of chromosomal subunits have recently been raised by Heddle.[7] He calculated that erroneous identification of the respective exchange classes might arise through both limitations of autoradiographic resolution and incorrect identification of "sister" chromosomes in the polyploid cells. He points out that matching of "sister" chromosomes may have been done in such a way as to maximize the frequency of twin exchanges. This combined with an autoradiographic resolution limit of 0.5–1.0 micron[9] in the light microscope leads to the misclassification of single exchanges as "false" twins. Inclusion of "false" twins would lead to incorrect twin:single ratios. Heddle[8] has concluded that there are alternative models, not involving subunit dissimilarity, of exchange formation that give predicted twin:single ratios that agree reasonably well with the adjusted existing data. It would seem, therefore, that the arguments for directional polarity of the subunits involved in exchange formation are not necessarily correct.

This communication offers an experimental test for the proposition that segregating chromosomal subunits are dissimilar. The test does not involve the identification of twin or single sister-chromatid exchanges, but it provides an independent evaluation of the conclusions which stemmed from this method.

Materials and Methods.—A clonal derivative of the Chinese hamster tissue-culture cell line CHEF-125 was used in all of these experiments. The cells were grown on Puck's fibroblast medium (PFM) supplemented with 15% fetal calf serum. All cultures were incubated at 36°C in a 5% CO_2-in-air atmosphere. Cultures were plated in Falcon plastic tissue-culture Petri dishes at a cell concentration of 250 cells per mm^2. When the cells attained log-phase growth, they were pulse-labeled with tritiated thymidine (^{3}H-TdR; 0.1 μc/ml; S.A. 1.9 c/mmole) for 6 hr. Immediately after removal of the isotope, the cultures were irradiated with 200 r of X rays; 1 hr later, colcemide (1×10^{-7} *M*) was added to and left in the cultures. Beginning 24 hr later, successive samples of C-metaphase figures were collected until a high incidence of polyploidy was observed. Cells were collected by scraping the Petri dishes and suspending the cells in Hanks' balanced saline solution (BSS). After two washes, the cells were suspended in a dilute (3 H_2O:1 Hanks' BSS) saline solution to effect hypotonic swelling. The cells were then fixed in 3:1 (methanol:acetic acid). Autoradiograms were prepared with Ilford L-4 liquid emulsion and Kodak D-19 developer.

Results.—Figure 1 summarizes the experimental theory: Following incorporation of the isotope, each chromatid will contain one labeled and one unlabeled segregating subunit. Proximal union following isochromatid breakage induced at this time will result, after suppression of anaphase with colcemide, in a "mirror-image" dicentric chromosome in the succeeding cell division. The pattern of label segregation in the dicentric will depend on whether there is a restricted rejoining of the breaks. Restricted rejoining may be of two types: (1) labeled-to-labeled and unlabeled-to-unlabeled association of the subunits will result in a dicentric having all the label conserved in one sister chromatid in the portion between the two centromeres, barring sister-chromatid exchange (Fig. 1*A*); (2) labeled-to-unlabeled association of the subunits will result in dicentrics

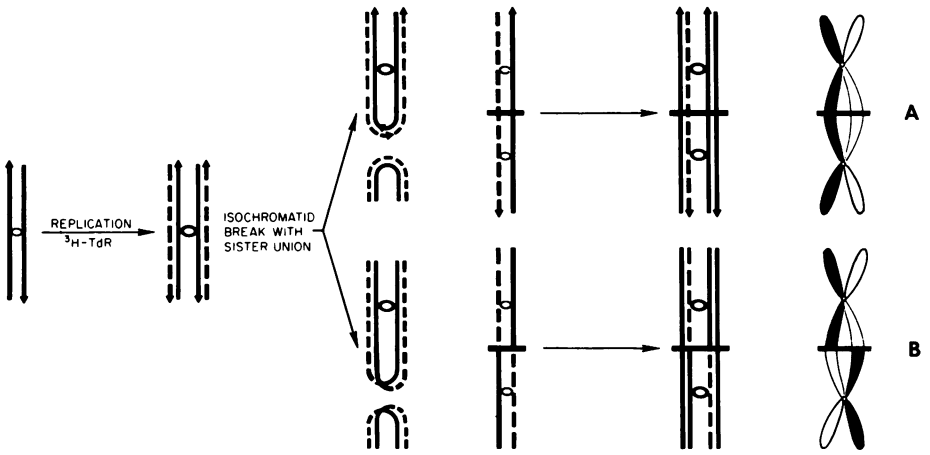

FIG. 1.—A diagrammatic representation of the model discussed in the text, showing the two alternate types of end-to-end association of the segregating chromosomal subunits. For simplicity, the subunits are represented as the helices of a single DNA molecule. The dotted line indicates the presence of ^{3}H-TdR, and the solid chromosome regions indicate presence of label.

all with a switch of label at the midpoint between the two centromeres (Fig. 1*B*). Random association of the subunits will yield equal frequencies of these two label patterns.

Several thousand polyploid cells were screened for the presence of mirror-image dicentrics generated from either easily distinguishable acrocentric and submedian-marker chromosomes or the four largest metacentric chromosomes of the Chinese hamster cells. Figure 2*A* shows a polyploid cell containing a dicentric generated from the submedian-marker chromosomes. After positions of the cells were recorded, autoradiograms were made for analysis of the label pattern. Interchromosome dicentrics were not expected to influence the data, since they occur in low frequency and since the analysis was restricted to those chromosomes in which proximal union dicentrics would have a different appearance from interchromosome dicentrics.

Analyses of 137 "mirror-image" dicentrics from polyploid cells were made. In 104 of them, all the label was conserved in one sister chromatid in the intercentromeric region. In 27 of the remaining 33 chromosomes, the label switches were in positions obviously distinct from the midpoint and were therefore considered examples of labeled-to-labeled subunit rejoining associated with a sister-chromatid exchange that occurred either before or after the formation of the dicentric (see Fig. 2*B*).

The remaining six chromosomes appear to have a label switch at their midpoint. These chromosomes may have resulted from labeled-to-unlabeled union of subunits but they can be accounted for on the restricted rejoining model, since with the observed frequency of 0.02–0.03 exchanges per micron of chromosome length, 3–8 of a sample of 137 chromosomes can be expected to have midpoint exchanges. In this calculation the midpoint is defined by the limits of auto-

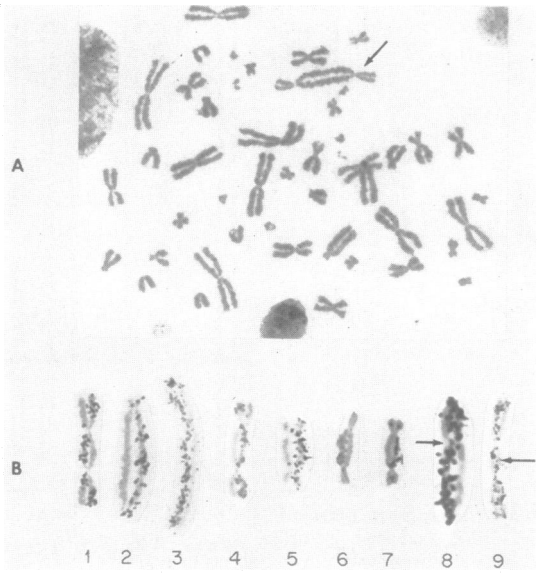

FIG. 2.—(*A*) Polyploid cell showing a dicentric involving the one marker chromosome.

(*B*) Representative examples of the label patterns observed in the dicentric chromosomes. Label switches are indicated by arrows. Dicentrics *2*, *6*, and *8* involve marker chromosomes. Examples *1–7* show no label switch in the intercentromeric region, whereas *8* has a switch just beyond the midpoint, and *9* has a midpoint switch. The large variation in chromosome size is due to variations in the degree of photographic enlargement.

radiographic resolution, viz. between 0.5 and 1 μ on either side of the true midpoints.[9]

Discussion.—The results are consistent with the assumption of a restriction in rejoining of chromosome subunits such that the labeled subunit of one chromatid is able to join with only the labeled subunit of its sister chromatid. This restriction is in accord with that imposed by the directional polarity of the single polynucleotide chains of DNA. Alternatively, since it has been adequately demonstrated that the newly synthesized strands of chromosomal DNA occupy an outside position with reference to the centromere in metaphase diplochromosomes,[3–5, 10] the labeled-labeled restriction might result from a pattern of outside-outside: inside-inside rejoining, imparted by conditions of "chromosome geometry." But the outside-outside "geometrical restriction" cannot be reconciled with the occurrence of sister-chromatid exchange in the first division after incorporation of label,[2] because such a restriction requires the reunion of a labeled strand to a labeled strand, and hence the absence of exchange-label segregation after a first division exchange (see Fig. 3*A*). The polarity model in the case of sister-chromatid exchange dictates labeled-to-unlabeled subunit association and, therefore, label segregation; it dictates only labeled-labeled subunit association in the case of proximal union (cf. Fig. 3*B*). Since it is known that sister-chromatid exchanges do occur in the first division,[2–4] "geometrical restriction" can be

postulated only if it is supposed that the restriction applies to proximal union configurations, but not to sister-chromatid exchange.

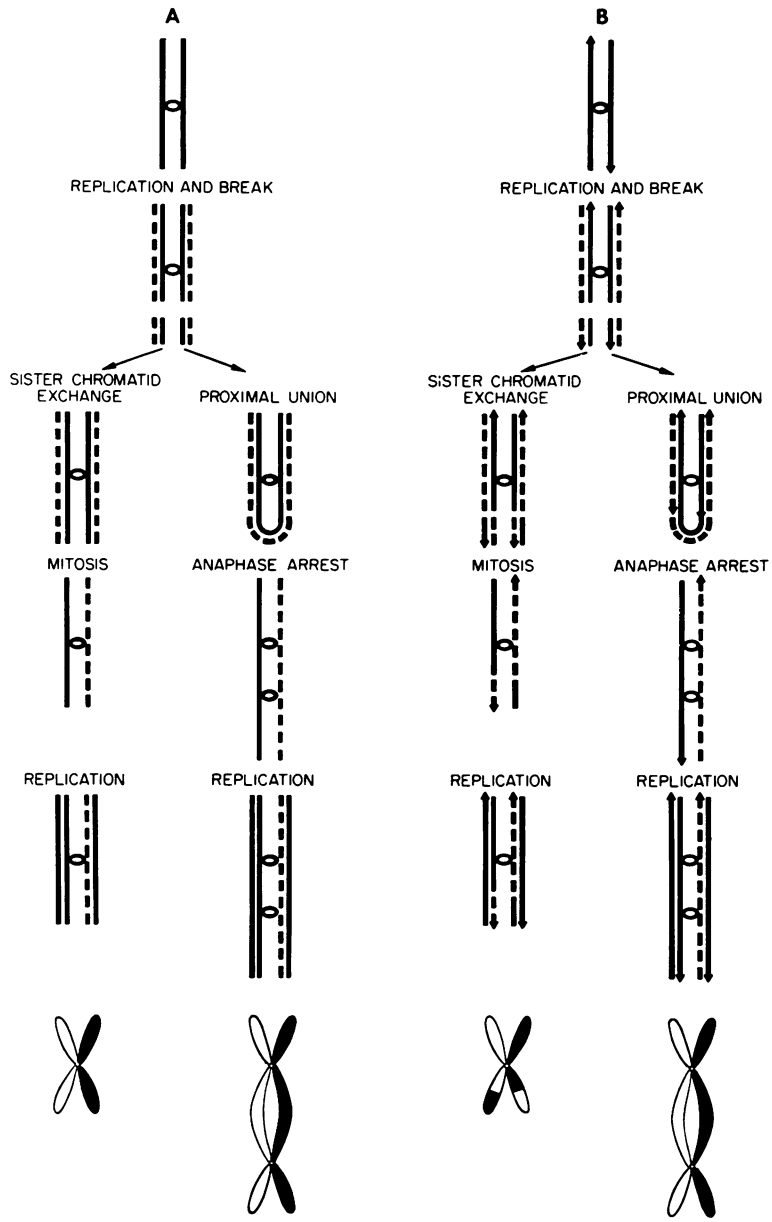

FIG. 3.—(*A*) Diagrammatic representation of label patterns expected for sister-chromatid exchange and proximal union if subunit reunion is determined by "geometrical restrictions." (*B*) Diagrammatic representation of label patterns exepcted for sister-chromatid exchange and proximal union if subunit reunion is restricted by directional polarity.

Summary.—Autoradiographic analysis of dicentric chromosomes generated by iso-locus breakage, followed by proximal union and subsequent induction of polyploidy, shows that the end-to-end rejoining of the segregating chromosomal subunits is not random. Our data are consistent with the assumption that directional polarity of the polynucleotide strands of DNA restricts the patterns of reunion.

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